

HISTOPHYSIOLOGICAL STUDIES ON THE CORPUS ALLATUM OF LEUCOPHAEA MADERAE. II. THE EFFECT OF STARVATION ¹

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The corpora allata of *Leucophaea maderae* undergo marked histological changes in conjunction with various phases of the reproductive cycle (Engelmann, 1957; Scharrer and von Harnack, 1958). Activation of the corpus allatum is characterized by an increase in organ volume, in nuclear number, and in absolute and relative cytoplasmic content. These changes, as well as the cytological manifestations of secretory activity, reach a peak shortly before ovulation. During the subsequent period of pregnancy, when the ovaries presumably receive no hormonal stimulation, the corpora allata return to the inactive condition (small size, dense nuclear arrangement) in which they remain until the onset of the next reproductive cycle.

The decision as to whether or not the corpora allata become activated at any given time resides in the central nervous system which, under certain conditions, exerts a restraining influence on these glands. In addition, nervous or neuro-humoral stimuli appear to be necessary to sustain the activity of the corpora allata (Scharrer, 1952; Engelmann, 1957). The type of message sent to the corpora allata is determined by a variety of afferent impulses from the external and internal milieu (see Scharrer, 1958, 1959). One of these is the nutritional state of the animal.

During a period of total starvation egg development is suppressed in *Leucophaea* (Scharrer, 1946); however, the ovary remains capable of responding to implanted active corpora allata (Johansson, 1955). From this result one can conclude that the absence of nutrients acts as a stimulus to the brain eliciting an inhibitory message to the corpora allata.

It was of interest, therefore, to examine the effects of inanition on the morphology of the corpora allata of adult females of *Leucophaea maderae* (von Harnack, 1958). The present study is concerned with two aspects of this problem: (1) Does prolonged total starvation affect the structure of the corpora allata, either to or perhaps beyond the point of preventing their activation? (2) How do corpora allata, kept inactive by starvation for a considerable period, respond to the resumption of a normal diet?

MATERIAL AND METHODS

The material on which this investigation is based consists of two series (A and B), one in which the animals were subjected to total starvation for various periods

¹ Supported by a Research Grant from the U. S. Public Health Service administered by Dr. Berta Scharrer.

² Grantee of the American Association of University Women.

of time, and another in which a period of starvation was followed by return to a normal diet. All animals used were adult females removed from the stock colonies on the day of emergence and isolated in pint-size jars. At the same time the period of starvation was initiated during which the majority of animals received only water; a small number was deprived of water as well as food.

Series A was used for the study of the influence of prolonged total starvation on the morphology and function of the corpora allata. It consisted of 52 females on a water diet, and of 31 females starved and dehydrated at the same time. The animals were fixed at intervals ranging from 5 to 95 days following the beginning of the starvation period, *i.e.*, the specimens surviving longest had reached an adult age at which normally the first reproductive cycle would have been completed. Animals showing increasing signs of weakness before being fixed could be presumed to have largely exhausted their nutritional resources. As might be expected, the point of exhaustion was reached sooner in the dehydrated group.

Series B gives information on the response of the corpora allata to the resumption of a normal food intake following an extended starvation period. In this group 51 newly emerged females received nothing but water for 30 days. They were then returned to a regular diet of dog chow and apple; they were kept together with normal males, and allowed to survive for up to an additional 90 days. In both experimental series, fixation was scheduled at five-day intervals, except during periods when more pronounced structural changes of the corpora allata were observed and, therefore, one- or two-day intervals became desirable.

Autopsies were performed in all cases in order to ascertain the condition of the reproductive organs. The histological procedure, and the method of quantitative evaluation of the corpora allata were the same as reported in the preceding paper of this series (Scharrer and von Harnack, 1958).

RESULTS

Series A: Starved animals

In contrast to the corpora allata of normally fed females which reach a four-fold increase in volume within 30 days after emergence, the corpora allata of starved females are noticeably suppressed (Fig. 1 and Fig. 3). The situation in the group of 52 animals kept on a water diet was as follows. During an initial period of 15 days, when inanition had not yet become effective, the corpora allata grew at a rate comparable to that in normally fed animals, *i.e.*, their volume doubled. After that the corpus allatum volume of starved females showed a gradual but continuous decrease. At 30 days of adult age the corpora allata had returned to the level of the newly emerged female, and the longest survivor, fixed after 95 days, had reached a minimal corpus allatum volume of 1.7 million μ^3 . This means that prolonged starvation had caused a decrease of corpus allatum volume to about one-half of the minimal size found in normally fed adult females.

The initial rise in corpus allatum volume of the starvation series is paralleled by an increase in nuclear numbers which compares with that in the normal control series. The turning point at which the nuclei begin to decrease in number occurs sooner in the experimental group (Fig. 1) than in the controls. Subsequently the nuclear counts return to a value characteristic of the normal inactive gland, while the cytoplasmic content drops considerably below the normal baseline. The

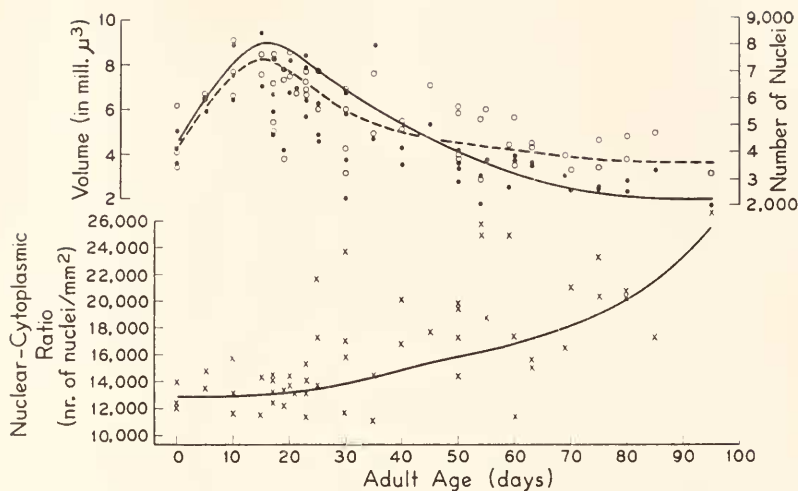


FIGURE 1. Diagram indicating morphological changes in the corpora allata of adult females of *Leucophaea maderae* which received only water from the day of emergence. Note that the volume of the corpora allata (—●—●—) and their number of nuclei (----○----○----) fall after a moderate initial rise. —x—x— = nuclear-cytoplasmic ratio. For comparison with normal controls see Figure 3.

result is a very crowded nuclear arrangement in the corpora allata of specimens fixed after excessively long periods of starvation.

These stepwise histological changes are enhanced in the starved-dehydrated group. When combined with dehydration, the first manifestations of inanition become evident earlier; the turning point in corpus allatum volume occurs already at about 12 days of adult age and appears accordingly somewhat lower on the curve (Fig. 3) than that in the starved-hydrated group. Thus the decline in corpus allatum volume which follows the moderate peak in the dehydrated group runs roughly parallel to that in the hydrated group and reaches the endpoint sooner (Fig. 3). Another indication of the aggravating effect of dehydration on starving animals was the extreme crowding of nuclei which made quantitative estimates of reasonable accuracy impossible. Nuclear counts were, therefore, omitted in the starved-dehydrated group of animals. In the present as well as earlier experimental series survival rates were consistently lower in starved-dehydrated than in starved-hydrated groups. The longest survival recorded in the dehydrated group was only 65 days.

As was to be expected from preceding studies (Scharrer, 1946; Johansson, 1955), none of the females deprived of food from the day of their emergence showed signs of reproductive activity. On autopsy, only small undeveloped eggs were observed in the ovaries, and the accessory sex glands contained no appreciable amount of secretory material.

Series B: Starved and re-fed animals

In this group of females the return to a normal diet occurred after a starvation period of 30 days in which only water was available; *i.e.*, at a time when the

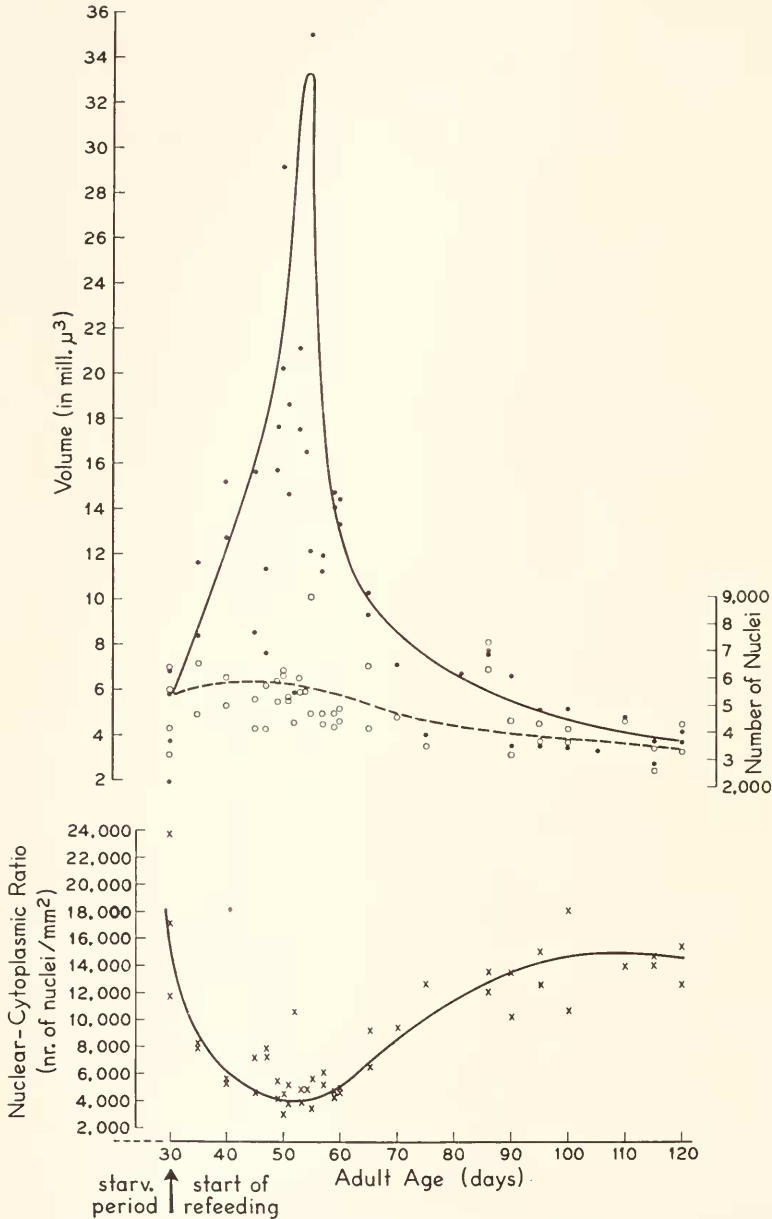


FIGURE 2. Diagram showing the response of the corpora allata in adult females of *Leucophaea maderae* which were starved, with access to water, for 30 days following emergence and were then returned to a normal diet. —●—●— = volume of both corpora allata; ----○----○---- = number of nuclei of both corpora allata; —×—×— = nuclear-cytoplasmic ratio (number of nuclei/ mm^2). Compare volumetric changes with those of normal controls (Fig. 4).

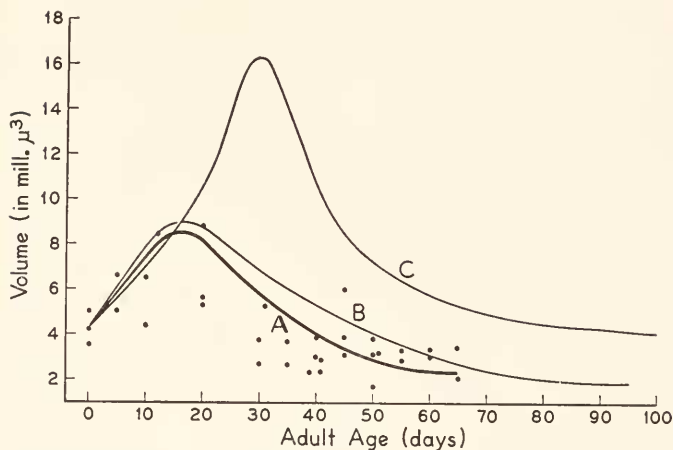


FIGURE 3. Diagram illustrating changes in the volume of both corpora allata of females of *Leucophaea maderae* which were starved and dehydrated following emergence (curve A). Compare with group of starved-hydrated animals (curve B) and with normally fed control group (curve C).

corpus allatum volume as well as the nuclear counts had almost returned to baseline levels characteristic of the normal inactive gland. Re-feeding resulted in a rather dramatic response of the corpora allata. Within 15–20 days, a peak was reached which represented a seven-fold increase in volume (Fig. 2). This considerably surpasses the peak characteristic of the growth curve of the first reproductive cycle under normal feeding conditions (four-fold increase in volume). It compares favorably with that of the second normal cycle which it also resembles with respect to the steepness of the slopes (Fig. 4). It appears as though the starved animal, on resumption of feeding, is capable of “making up for lost time” and does not have to start its reproductive period as “gradually” as the normal animal.

The rapid rise of corpus allatum volume in the starved and re-fed series is not accompanied by a correspondingly high increase in nuclear number (Fig. 2). Consequently, the largest corpora allata have an exceptionally high content in cytoplasm, and accordingly the nuclei are more widely spaced than in the most active glands of the first reproductive cycle of the normal series.

The full-scale activation of the corpora allata in this first reproductive cycle of the starved-re-fed animals promptly led to stimulation of the ovaries; ovulation occurred within about 25 days after the resumption of feeding.

DISCUSSION

In the present as well as earlier experiments (Scharrer, 1946; Johansson, 1955; Willis and Lewis, 1957), the roach *Leucophaea maderae*, when subjected to total starvation, showed a considerable capacity to survive. If water was provided, adult females which were starved from the day they emerged lived for up to three months at room temperature. This period was shortened by about three weeks, when the insects were deprived of water as well as solid food. In *Leucophaea maderae*, as in certain other insect species, eggs do not develop in the total

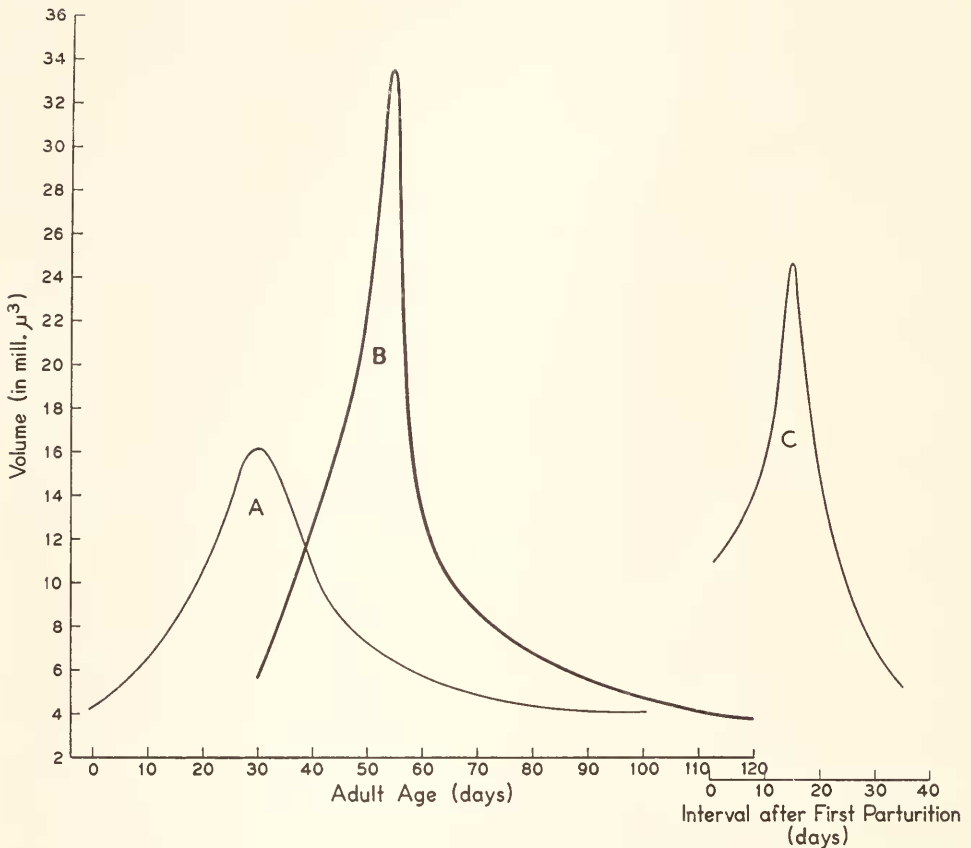


FIGURE 4. Diagram showing the pronounced rise in corpus allatum volume of starved-re-fed females (curve B), in comparison with corpus allatum growth in first normal (curve A) and second normal reproductive cycle (curve C).

absence of nutrients (Scharrer, 1946), unless active corpora allata are implanted (Johansson, 1954; 1955), or the starving insect's own corpora allata are released by surgery from the action of the restraining nerves (Johansson, personal communication). These results demonstrate that (a) the gonads of starved animals have the capacity to respond to gonadotropic stimulation, and (b) the initial moderate rise in corpus allatum volume observed in starving *Leucophaca* females seems to represent a degree of activation insufficient to elicit ovarian response.

The longer the period of inanition, the smaller and the poorer in cytoplasmic content are the corpora allata. It is difficult to estimate the number of nuclei present in these small organs, and to demonstrate possible signs of secretory activity. At the endpoint, beyond which survival was no longer possible, the corpora allata had only about one-half the volume of normal female "inactive" glands. It cannot be determined with certainty whether or not this decrease below the normal range of corpus allatum size is nothing more than a general effect

of inanition shared by other organs of the body. An attempt was made to measure organs in the vicinity of the corpora allata, such as the musculature of the head. A comparison of the diameters of muscle fibers in normal and starved specimens suggests that some "wasting" occurs in the latter. This is also evident from the larger spaces between muscle fibers in drastically starved animals. However, since the individual muscle elements vary in width, a reasonably accurate estimation of the degree of shrinkage is not possible.

What was said so far applies to starved animals receiving water, and to an even larger degree to starved-dehydrated specimens. Their corpora allata do not even reach the size of the starved-hydrated group, and the decline in volume (and activity) occurs proportionately sooner. A comparable dependency of the morphology and function of the corpus allatum on the nutritional state has been observed also in several other insect species (Wigglesworth, 1936; Schwinck, 1951; Müssbichler, 1952; Engelmann, 1957).

In a general way, the effects of nutritional deficiency on the corpus allatum of the insects compare with those on its analogue, the anterior pituitary of the vertebrates.

A variety of studies in mammals have shown that, under conditions of starvation, (a) distinct structural and functional changes occur in the pars anterior, (b) gonadal malfunction is attributable to suppression of gonadotropic activity; and (c) the reproductive system deficient because of starvation responds to the administration of pituitary material.

The morphological changes observed in pituitaries of starving laboratory mammals as well as human patients, such as decrease in the volume or weight of the anterior lobe (Jackson, 1917), in the size and number of parenchymal (acidophilic) cells (Sedlezky, 1924; Schubothé, 1940; D'Angelo *et al.*, 1948), and in relative cytoplasmic content (Jackson, 1917), are in line with the changes described in the present study for the corpora allata of the insect, *Leucophaea*.

As in the insect, the concomitant disturbance of gonadotropic activity in the mammals studied led to depression of gonadal function (Mulinos and Pomerantz, 1940; Rinaldini, 1949/50) which could be remedied by the administration of gonad-stimulating substances (Boutwell *et al.*, 1948; Rinaldini, 1949/50).

It was of particular interest to observe the effects of the return to a normal diet in females of *Leucophaea* whose ovarian activity had been restrained by a 30-day starvation period following emergence. Instead of beginning their reproductive period in the same manner as young normal adults, even though belatedly, starved-re-fed females "skipped the first cycle" for which a slower and more moderate activation of the corpora allata is typical. At once they acted at full capacity. Thus, the return to ample food supplies may represent a powerful stimulus for corpus allatum growth. Within a short period of 20–25 days an up to seven-fold volumetric increase occurred. The subsequent decline from these high values was equally rapid. From every point of view, the curves illustrating this reproductive cycle do not differ essentially from those characteristic of the second or subsequent cycles in normal specimens (Figs. 2, 4). In fact, the peak illustrating the range of corpus allatum activation is higher in the starved-fed group than in any cycle of our normal series. However, this difference might perhaps be due to individual variation, since Engelmann's (1957) values for the second reproductive cycle of

normal females reach approximately the same maximum as that obtained in the present series of experimental animals. On the other hand, the values obtained by Engelmann are not entirely comparable to those reported here, because his experiments were conducted under different conditions of temperature and humidity. At any rate, there can be no doubt that corpora allata of animals having been starved for some time and then returned to normal food supply, respond with great readiness and display pronounced signs of activation. The same observation was made in nymphs of *Panorpa* (Schwinck, 1951). In contrast to a newly emerged animal, a starved-fed female undergoing its first reproductive cycle has had time to adjust to the changes connected with "metamorphosis." This may be the reason why its corpora allata, like those of older normal specimens, respond maximally as soon as the restraining effect of the brain is lifted. This would mean that the "post-emergence maturation" postulated by Rockstein (1956) can take place under conditions of total starvation.

Be this as it may, within certain limits the degree of corpus allatum activation obtained under various normal and experimental conditions is perhaps not too significant. One must keep in mind that the more moderate activation of the corpora allata in the first normal cycle suffices for the development of a full set of eggs. The more pronounced response of the corpora allata in more mature adults, normal as well as starved-fed may be the result of a higher metabolic rate of their tissues. This possibility is suggested by comparable data in mammals.* Here, re-feeding after starvation resulted in an increased metabolic rate (Quimby *et al.*, 1948) and in a prompt response of the anterior lobe (Jackson, 1917).

SUMMARY

1. Adult females of *Leucophaea maderae* were subjected to total starvation, with or without dehydration, following their emergence. Throughout the period of survival, *i.e.*, up to 95 days, no egg development occurred as a consequence of the failure of the corpora allata to become properly activated. The corpora allata of a series of starved animals fixed after varying intervals showed only a small initial rise in volume which was followed by a gradual decrease reaching a minimum below that of normal controls.

2. In another series of animals, starved for 30 days, the return to a normal diet promptly initiated a growth phase in the corpora allata which considerably surpassed that characteristic of the first reproductive cycle in normal animals (seven-fold, instead of four-fold volumetric increase). As to speed and degree, this period of activation compared favorably with that of the second cycle in normal females. Thus the delay in reproductive activity, caused by starvation, was at least in part compensated for by a more rapid and complete activation of the corpora allata which in turn promptly led to ovarian development.

3. The effects of starvation and re-feeding on the structure and function of the corpora allata of *Leucophaea* are in line with those described in the literature for the analogous organ in mammals, *i.e.*, the anterior lobe of the pituitary.

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